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ART. VI.—Association of Alga and Fungus in Salmon Disease.

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[Read 12th May, 1910].

In a short paper communicated to the Royal Microscopical Society, in 1907, by Dr. Hebb, I drew attention to the growth of an Alga—Myxonema tenue (Ag) Rabenh.—on Carassius auratus, found in a garden tish-pond in Melbourne.¹ Incidentally the occurrence of a fungus and a number of unicellular algae was noted. This report was responded to by Kumagasu Minakata, who described an algal growth on small fry of Haplochilus latipes, Schley, in a shallow bog pool of the Asso Marsh, Tanabe, Japan, and the Alga, identified in England by George Massee, proved to be the same species as that recorded by me in the foregoing case, while with it were found fragments of a diatom of the genus Gomphonema and a damaged individual of Euastrum sp. Neither Minakata nor Massee mentioned the presence of a fungus.²

I purpose giving a further illustration of what is, at least sometimes, an epipiscal alga, and, as the occurrence affected a large number (about 10 per cent.) of fish in the Studley Park ponds at Kew, Melbourne, the matter is raised to one of conomic importance, and it may be desirable to describe the circumstances under which the trouble arose. In an appendix I offer for record the names of a number of Algae, either new for Victoria or interesting in this connection.

The new fish ponds in Kew were, less than a year ago, stocked from the ponds of the Zoological and Acclimatization Society's supply at Royal Park, where the fry had been hatched from ova imported from New Zealand, under the direction of D. Le Souëf. There is no reason for supposing that the ova

¹ Jour. R.M.S., 1907, pp. 279-281.

^{2 &}quot;Fish Infested with Alga." Nature, Nov. 26, 1908.

may have been unhealthy, as the fry raised in the Zoological Gardens have not suffered from disease, though a few died, thinks Mr. Le Souëf, from overcrowding. In the Studley Park ponds now under notice, brown, rainbow and Lochleven trout are being reared. These are respectively *Salmo fario*, *S. irideus* and *S. levenensis*.

The ponds comprise six excavations measuring about 14 metres long x 5 m. broad; depth of water about 1.5 m. Each receives its own supply, and has a separate outlet of water. The supply is from the Yarra Yarra River, whence it is pumped up to a circular masonry reservoir in Studley Park. From the reservoir, which is close to the ponds, the water is conveyed by underground pipes to the pond's enclosure, thence by open channels in the ground, and in open wooden flumes. The pump which is used for filling the reservoir with river water is near to and below the weir at Dight's Falls, and the river, above and below, is polluted mainly by drainage from the populous suburbs on the basaltic flats on the opposite side. Though the sewerage works extension has greatly reduced the evil, there is still much that is injurious entering the river by way of Reilly-street and other drains, Merri Merri Creek, Darebin Creek, etc.

Conditions inimical to the welfare of young fry, which had hitherto rejoiced in the purity of the metropolitan domestic supply of water from the Yan Yean and Maroondah systems, were obtained during the prolonged, fierce heat of the Christmas and New Year holidays, when pumping operations partly ceased, and the water in the reservoir fell to a low level. The reservoir, used for irrigating public gardens, and for hydraulic elevators, at times contained a considerable amount of silt. In addition to the direct rays of the sun, a great amount of heat was reflected from the face of the surrounding stonework. The pipes from the reservoir were not deeply buried, and the hard ground was fairly warm during the hot weather. Thus the temperature of water in the ponds rapidly rose until a maximum of 77 degrees Fahr, was reached during an air temperature of about 100 degrees in the shade.

The first sign of trouble was the appearance, near the surface, of rainbow fry of sluggish habit, and these became gradually more enfeebled until death ensued. Brown and Lochleven fry were also affected, but not to the same extent. On many of the fish there appeared tufts of vegetable growth similar to those noted by myself in Victoria, and by Minakata in Japan, the branched filaments of *Myronema* being about 1 cm. long and attached to almost any part of the body, but mostly to back and sides. The fungus previously referred to was present, and I am satisfied that it is at least a member of the Saprolegniaceae, if not a species of *Saprolegnia*. Amongst the filaments of these composite tufts there were other algae, both multicellular and single-celled plants, some of the latter being motile.

While many of the weak and dying fish bore no conspicuous growth, and showed on closer examination no fungus mycelium, they were still in a diseased condition, disorganisation of the mouth parts, gills, fins and other portions of the body being easily seen, the scales having been lost from the affected areas.

Sometimes the Saprolegnia appeared alone, but usually, if not always, at spots where ulcerations were in evidence. I was unable to find any attached Myronema apart from the fungus. It is possible that in the case quoted by Harz¹, not necessarily an enzyme, but probably a bacillus prepared the way for the fungus mycelium. On the other hand, Professor Huxley² in sisted that this view of Saprolegnia, which marked it as a Saprophyte, was incorrect, and gave an account of experiments by which he was able to demonstrate the gradual encroachment of the mycelium threads, from an infected part, on sound tissue of the epidermis, which they destroyed in their progress. In view of the absence of visible fungus under ordinary microscopic examination in present case, I put forward the suggestion that a bacillus such as B. salmonis pestis' or B. piscicidus bipolaris⁴ prepares the tissue for the Saprolegnia, which is then a Saprophyte, but that it may in the absence of such a bacillus adapt itself to other conditions, and then as a true parasite dissolve the tissues for itself by means of an enzyme.

¹ Jour. Roy. Micr. Soc., 1907, p. 201.

² Report, Fisheries Exhibition Literature, vol. vi., 1882.

³ Rep. British Association, 1902.

⁴ Proc. Linn. Soc. N.S. Wales, vol. xxv., 1900, pp. 122-130.

Some tadpoles succumbed to the disease, and these had the same disorganised mouth parts, etc., but no visible *Saprolegnia* or Alga, and therefore, keeping in mind J. Hume Patterson's statement¹ that *B. salmonis pestis* is not pathogenic to frogs, it seems probable that *B. piscicidus bipolaris*, Greig, would be found on bacteriological investigation.

The presence of Myronema tenue appears to depend on the preceding growth of the fungus, in the mycelium of which zoospores of the former may be enmeshed. In the Yarra River, above Dight's Falls, this species occurs as long streamers in the current, attached to willow, twigs, etc. In the wooden flumes of the Kew fish-ponds it attained in January a length of 22 cm., and in the ponds there was a sickly growth, the poor development being evident both in reduction of size of the plants and richness of colour of chloroplasts, etc. The plants on the fish, though very small, were vigorous and rich in colour, while some of the branchlets were actively producing zoospores at time of collection. The suggestion made in my previous paper that this stream-loving plant adapts itself to comparatively stagnant water by securing a foothold on a motile substratum, seems to hold good.

The Fisheries Branch of the Ports and Harbours Department lost no time in constructing sunshades, and by thus lowering the temperature and by cleansing the reservoir, which will probably be kept at high level, has reduced the trouble in the course of a few months almost to the vanishing point.

One of Huxley's determinations, in 1882, was that the facts obtained in his investigations were not favourable to the supposition that either pollution or overcrowding had much to do with the matter, and again, that we could not make progress until the relationship between the sporadic and epidemic phases of the disease, as affecting salmon, became known. The mention of salmon disease in the Fraser River, British Columbia, and in the Castries River, Siberia, does not, for climatic reasons, affect the present case. I have endeavoured to show that, given pollution and high temperature, there is great danger, and that the trouble has been almost removed by reducing these to normal conditions.

¹ Loc. cit.

APPENDIX.

LIST OF ALGAE.

[* New Record for Victoria.]

1	-	~	From the Yarra River at Pumping Station.
2	-	-	From Zoological Gardens Fish Ponds. (Water from
			the Yan Yean and Maroondah Systems).
3	-	-	From the earth gutters of the fish ponds at Kew.
4	-	-	From the wooden flumes at the fish ponds at Kew.
5	-	-	Removed from the fish.
6	-	-	In some ferruginously-coloured spring water about
			2 cm. deep, among grass, bearing an iridescent

scum; fish ponds, Kew.

Date—From January to May, 1910, excepting 1 and 5 noted for January only.

CHLOROPHYCEAE.

) -	()ec	logonium	sn
 ., .	·	See	Somerin	Sp.

- 3, 4, 5 Myxonema tenue (Ag.), Rabenh.
- 3 - Mougeotia sp.
- 3 - Zygnema sp.
- 2 - Closterium Ehrenbergii, Menegh.
- ^{*}2 - Closterium moniliferum (Bory), Ehrenb.
- 3 · Closterium Dianae, Ehrenb.
- 6 - Closterium striolatum, Ehrenb.
- 4 - Closterum sp.
- 2, 3 Pleurotaenium coronatum (Breb.), Rabenh.
- 3 - Micrasterias Mahabuleshwarensis, Hobs., forma.
- *3 - Euastrum Turnerii, West.
- *5 - Penium libellula (Focke), Nordst.
- 5 - Netrium digitus (Ehr.), Itzigs and Roths.
- 5 - Desmidium Baileyi (Ralfs), De Bary, forma.
- 2 - Cosmarium Hardyi, G./S. West.
- 3 - Cosmarium reniforme, Ralfs (?).
- 3 - Cosmarium sp.

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 Pediastrum duplex, Meyen, var. reticulatum, Lagerh.
Kirchneriella lunaris (Kirch.), Möb.
Ankistrodesmus falcatus, var. acicularis (A. Br.), G. S. West.
Scenedesmus quadricauda (Turp.), Bréb.
Characium Pringsheimii, A. Br.
Sphaerocystis Schroeteri, Chod.

BACILLARIEAE.

3,	5	-	Surirella robusta, Ehrenb.
*6	-	-	Eunotia pectinalis (Kütz), Rabenh.
3	-	-	Cocconema lanceolatum, Ehrenb.
*3	-	-	Synedra pulchella, Kütz.
3	-	-	Navicula sp.
õ	-	_	Gyrosigma elongatum, W. Sm.

MYXOPHYCEAE.

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3	-	~	Oscillatoria tenuis, Ag.
*3	-	-	Oscillatoria princeps, Vauch.
3	-	-	Phormidium tenue (Menegh.), Gom.